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14. ABSTRACT

The purpose of this research is to determine the association between ovarian cancer and (1) imbalances in estrogen metabolism that lead to higher levels of estrogen-DNA adducts in urine and/or (2) genetic polymorphisms in selected enzymes that metabolize estrogens. We completed this study as planned, demonstrating that estrogen metabolism is unbalanced in women diagnosed with breast cancer, as detected by the ratio of estrogen-DNA adducts to estrogen metabolites and conjugates (ratio = 91.4 ± 43.1) compared to the balanced estrogen metabolism in the healthy controls (ratio = 24.7 ± 12.7, $p<0.0001$). In addition women who were homozygous for the catechol-O-methyltransferase allele and the cytochrome P450 1B1 high activity allele had significantly increased DNA adduct ratios and increased odds of having ovarian cancer.

15. SUBJECT TERMS
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Table of Contents

Page

Introduction

Ovarian cancer is the fifth leading type of cancer in women in the U.S. [1,2], but first in gynecological cancer mortality [2]. Our inability to diagnose ovarian cancer at an early stage is a major problem. We hypothesize that ovarian cancer is initiated by imbalanced estrogen metabolism leading to estrogen-DNA adducts that generate mutations in critical genes in the ovarian epithelial cell [3]. If so, analysis of estrogen-DNA adducts could provide a tool for early diagnosis [3]. In this project we proposed to analyze 38 estrogen metabolites, conjugates and DNA adducts in urine samples [4] and genetic polymorphisms in four selected estrogen-metabolizing enzymes in DNA saliva samples from 50 women diagnosed with ovarian cancer and 50 matched controls. The goal of this project is to determine whether increased levels of estrogen-DNA adducts and/or specific genetic polymorphisms are associated with ovarian cancer.

Body

We proposed to meet the goals of this project by accomplishing the following specific aims:

1. Collect spot urine samples (50-ml) from groups of 50 healthy women not diagnosed with ovarian cancer and 50 women diagnosed with epithelial ovarian cancer, process aliquots by solid-phase extraction (SPE) and analyze the estrogen metabolites, conjugates and depurinating DNA adducts by using ultraperformance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS).

2. Analyze SNPs in CYP1A1, CYP1B1, COMT, and NQO1 in DNA recovered from saliva samples from the same subjects.

The relative estrogen-DNA adduct levels and frequencies of genetic polymorphisms were correlated with clinical and pathological variables. From these correlations we hoped to gain evidence on the utility of the estrogen-DNA adducts and/or enzyme polymorphisms to serve as biomarkers to screen for ovarian cancer.

Task 1. Obtain approval of the protocol from the OCRP Human Research Protection Office (Sp. Aims 1 & 2).

Completed in Year 1.

Task 2. Prepare to recruit subjects (100 total) into the study (Sp. Aims 1 & 2).

Completed in Year 1.

Task 3. Begin recruiting subjects (at least 50) into the study and collecting urine and saliva samples (Sp. Aims 1 & 2).

The efforts in Year 1 to increase subject enrollment were successful, but enrollment still was not adequate. Therefore, in Year 2, the inclusion criteria were expanded to include African-American women and additional cancers of the same origin. These changes were approved by the Department of Defense. In Year 1, we recruited 9 subjects. In Year 2, we recruited 29 more subjects for a total of 38. All of the urine and saliva samples were sent from Carolinas Medical Center to the University of Nebraska Medical Center (UNMC). In Year 3 we completed enrollment of subjects, although we did not reach the planned 50 cases and 50 controls. We enrolled a total of 34 cases that provided urine and saliva samples and completed the

questionnaire. We enrolled 2 cases that provided only a saliva sample and completed the questionnaire. We enrolled 36 age-matched controls that provided urine and saliva samples and completed the questionnaire.

An ACCESS database was created by Dr. Beseler in Year 1 for the subject questionnaire data. The database facilitates extraction for analysis and linking of matched cases and controls. Data from the 72 questionnaires have been entered into the database. A coding plan was developed in order to expedite analysis once the genotyping is completed and the urine samples are processed.

Task 4. Begin processing urine samples and analyzing them by UPLC-MS/MS (Sp. Aim 1).

Although we processed the urine samples as they were sent to UNMC, we chose not to analyze them until all samples were in hand, in order to minimize variations in the analyses. In the second year, our UPLC-MS/MS instrumentation required extensive refurbishing by Waters service technicians, and we spent several months resetting instrument parameters. In Year 3, the instrument worked at its peak performance, and we successfully analyzed all of the urine samples. The results are reported below.

Task 5. Begin purifying DNA from saliva samples (Sp. Aim 2)

We purifed the DNA from all 72 saliva samples. Almost all of the DNA samples were successfully analyzed, and the results are reported below.

Task 6. Order and test primers for analysis of the SNPs (Sp. Aim 2).

We ordered and tested the primers for analysis of SNPs in Year 3, and the SNPs were also analyzed.

Key Research Accomplishments

1. Obtained approval of the protocol.
2. Synthesized or purchased estrogen metabolite, conjugate and DNA adduct standards for UPLC-MS/MS.
3. Refurbished the UPLC-MS/MS instrumentation to achieve peak performance in analyses.
4. Optimized purification of DNA from saliva and purified DNA from the all 72 subjects..
5. Established an ACCESS database to analyze data obtained from subject questionnaires and entered and analyzed data from all 72 subjects.
6. Recruited 72 subjects and obtained urine and saliva samples.
7. Processed and analyzed all 70 (36 control and 34 case) urine samples by UPLC-MS/MS.
8. Processed and analyzed SNPs in all 72 saliva samples.
9. Statistically analyzed the results obtained for estrogen-DNA adducts, SNPS and demographic data.

Reportable Outcomes

The first and most exciting outcome from this study is the finding that women diagnosed with ovarian cancer have significantly higher amounts of estrogen-DNA adducts than age-matched women who have not been diagnosed with cancer (Fig. 1). The level of estrogen-DNA adducts in urine samples was measured as the ratio of estrogen-DNA adducts to estrogen metabolites and conjugates (see ratio in Fig. 1). This ratio provides a measure of the imbalance

of estrogen metabolism in a person. A high ratio indicates that the person's estrogen metabolism is unbalanced, leading to formation of higher levels of catechol estrogen quinones, which react with DNA to form adducts. A low ratio indicates that a person's estrogen metabolism is balanced, and formation of estrogen-DNA adducts is relatively low.

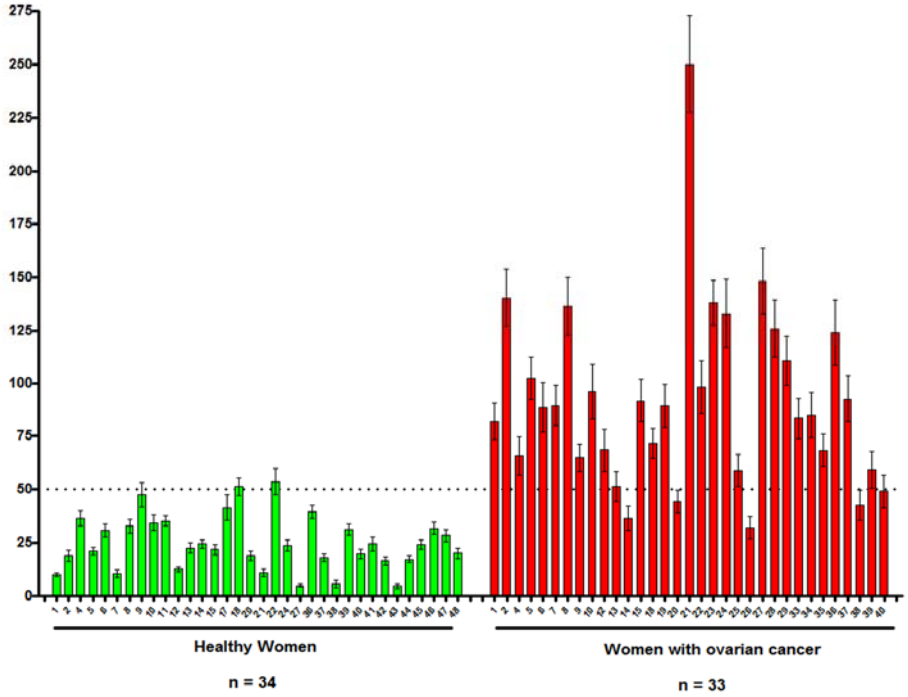

Figure 1. Ratios of depurinating estrogen-DNA adducts to estrogen metabolites and conjugates in urine samples from healthy control women and women diagnosed with ovarian cancer. The ratios were significantly higher in cases ($p < 0.0001$).

$$\text{Ratio} = \frac{\text{4-OHE}_1(\text{E}_2)\text{-1-N3Ade} + \text{4-OHE}_1(\text{E}_2)\text{-1-N7Gua}}{\text{4-catechol estrogens} + \text{4 –catechol estrogen conjugates}} + \frac{\text{2-OHE}_1(\text{E}_2)\text{-6-N3Ade}}{\text{2-catechol estrogens} + \text{2-catechol estrogen conjugates}} \times 1000$$

The cases (n=33) had a mean ratio of 91.4 ± 43.1 and the controls (n=34) had a mean ratio of 24.7 ± 12.7, which was significantly lower ($p<0.0001$). These results can also been seen in Figure 2. Therefore, we achieved Specific Aim 1, the main objective of this project, by demonstrating that estrogen metabolism is unbalanced in women diagnosed with ovarian cancer, and estrogen-DNA adducts could serve as a biomarker of ovarian cancer.

Sample characteristics and risk factors for ovarian cancer. The sample was primarily white, married and well-educated. The cases of ovarian cancer did not differ by age due to having been age-matched, nor did they differ by age at menarche, body mass index and years having smoked cigarettes (Table 1). As has been reported previously in the literature [5], significant differences were observed for age at first birth (Cohen's D for effect size = 0.69, large effect) and years of oral contraceptive use (Cohen's D for effect size = 0.50, medium effect). The binary variable for ever having used oral contraceptives remained significantly protective against developing ovarian cancer (OR=0.22; 95%CI 0.05-0.90). These effect estimates are similar to what has been previously reported. Controls were likely to be older when they gave birth to their last child and tended to be older at the time of their last menstrual period. Nearly all the women were post-menopausal, few had ever used HRT, few had undergone a tubal ligation, and most did not have a family history of ovarian cancer. These risk factors did not provide the variability needed to detect differences in the odds of ovarian cancer. Most of the women reported being in good, very good and excellent health. Indicative of being a healthy sample of women, 66% of the women in this sample took a daily multivitamin and 75% took one or more antioxidants. The majority of women did not experience a natural menopause, but this did not differ between cases and controls.

Table 1. Demographic characteristics of 67 study participants with complete information for adduct ratio and genotypes and tests for significant differences between cases of ovarian cancer and controls.

Demographic characteristic	Entire sample (n=67) Mean (SD)	Controls (n=34) Mean (SD)	Ovarian Cancer (n=33) Mean (SD)
Age	58.3 (7.01)	58.4 (7.24)	58.1 (6.87)
Age of menarche	12.9 (1.79)	12.8 (1.77)	12.9 (1.84)
Age at first baby [c]	24.4 (5.75)	26.2 (5.29)	22.4 (5.67)
Age at last baby [a]	28.7 (5.69)	30.1 (5.51)	27.3 (5.63)
Years of oral contraceptives [b]	5.91 (6.87)	7.56 (7.53)	4.21 (5.74)
Age at last menstrual period [a]	47.1 (7.75)	49.2 (6.28)	45.3 (8.58)
BMI	28.8 (5.57)	28.3 (4.46)	29.3 (6.56)
Years of cigarette smoking	17.4 (11.0)	16.0 (9.73)	18.3 (12.0)
DNA adduct ratio [d]	57.5 (45.9)	24.7 (12.7)	91.4 (43.1)

[a] $p<0.10$; [b] $p<0.05$; [c] $p<0.01$; [d] $p<0.0001$

Differences in lifestyle factors were apparent in cases and controls, but are partially explained by differences in their educational attainment (Table 2). Controls had significantly more years of higher education than cases. Education was significantly associated with exercising briskly three or more days per week (p=0.007). Women who reported that they exercised less than twice a week and did not break a sweat were three times more likely to have ovarian cancer than women who exercise vigorously three or more times per week in a univariate model (OR=3.32; 95% CI 1.08-10.2). After adjusting for level of education, the odds were reduced but remained elevated (OR=2.33; 95% CI 0.71-7.67). Previous studies on the association between exercise and risk of ovarian cancer have been inconsistent with most finding a small protective effect or no effect. Body mass index was not acting as a confounder because there was no difference in body mass index between cases and controls (Table 1).

Although previous studies have generally not found an association between alcohol consumption and ovarian cancer, in this study women who did not drink alcohol were at a significantly higher odds of ovarian cancer (OR=4.88; 95% CI 1.39-17.1). Education was also significantly associated with alcohol consumption (p=0.009); women with education beyond high school were more likely to consume alcohol. After adjusting the association of current alcohol consumption to ovarian cancer by educational level, the odds of ovarian cancer remained elevated, but marginally significant (OR=3.48; 95% CI 0.93-13.0). The lack of significance is probably due to adding a second independent variable.

Only three women were current smokers, but women who reported ever having smoked cigarettes had an elevated risk of ovarian cancer (OR=2.59; 95% CI 0.95-7.05). Previous studies have been inconsistent in reporting an increased risk of ovarian cancer in women who have a history of cigarette smoking.

The DNA adduct ratio was significantly associated with having ovarian cancer. In adjusted logistic regression models, other significant risk factors became insignificant with the adduct ratio in the model. Additionally, the adduct ratio was not significantly associated with any other risk factors for ovarian cancer, suggesting that the results cannot be explained by confounding by another risk factor.

Table 2. Demographic characteristics of 67 study participants with complete information for adduct ratio and genotypes and tests for significant differences between cases of ovarian cancer and controls.

Demographic characteristic	Entire sample (n=67) N (%)	Controls (n=34) N (%)	Ovarian Cancer (n=33) N (%)
Race			
White	62 (92.5)	33 (97.1)	29 (87.9)
African American	5 (7.5)	1 (2.9)	4 (12.1)
Education [c]			
< = High school graduate	33 (49.3)	11 (32.3)	22 (66.7)
> High school graduate	34 (50.7)	23 (67.7)	11 (33.3)
Marital status			
Married	47 (70.1)	26 (76.5)	21 (63.6)
Not married	20 (29.9)	8 (23.5)	12 (36.4)
Employed [c]			
No	18 (26.9)	4 (11.8)	14 (42.4)
Yes	49 (73.1)	30 (88.2)	19 (57.6)
Post-menopausal			
No	3 (4.5)	3 (8.8)	0
Yes	64 (95.5)	31 (91.2)	33 (100.00)
Type of menopause			
Surgically-induced	42 (64.6)	21 (65.6)	21 (63.6)
Natural menopause	23 (35.4)	11 (34.4)	12 (36.4)
Health status			
Excellent, Very good, good	62 (92.5)	33 (97.1)	29 (87.9)
Fair or poor	5 (7.5)	1 (2.9)	4 (12.1)
Exercise frequency [b]			
> 2 days/ week	20 (30.3)	14 (42.4)	6 (18.2)
<1-2 days/week	46 (69.7))	19 (57.6)	27 (81.8)
HRT			
No	56 (83.6)	28 (82.3)	28 (84.8)
Yes	11 (16.4)	6 (17.7)	5 (15.2)
Oral contraceptive use [b]			
No	13 (19.4)	3 (8.8)	10 (30.3)
Yes	54 (80.6)	31 (91.2)	23 (69.7)
Parity			
No children	13 (19.4)	6 (17.7)	7 (21.2)
At least one child	54 (80.6)	28 (82.3)	26 (78.8)
Ever smoke cigarettes [a]			
No	28 (41.8)	18 (52.9)	10 (30.3)
Yes	39 (58.2)	16 (47.1)	23 (69.7)
Current drinker [c]			
Yes	50 (74.6)	30 (88.2)	20 (60.6)
No	17 (25.4)	4 (11.8)	13 (39.4)
Relative with cancer			
No	24 (35.8)	11 (32.3)	13 (39.4)
Yes	43 (64.2)	23 (67.7)	20 (60.6)
Relative with ovarian cancer			
No	64 (35.5)	34 (100.0)	30 (90.9)
Yes	3 (4.5)	0	3 (9.1)

[a] $p<0.10$; [b] $p<0.05$; [c] $p<0.01$; [d] $p<0.0001$

In summary, several of the strongest associations with ovarian cancer (age at first birth and oral contraceptive use) were replicated in this study of 67 women. Other associations may be due to the specific characteristics of this sample, such as higher educational attainment in the controls compared to the cases of ovarian cancer. The sample tended to be healthy, took vitamins and supplements, and more than half reported eating vegetables every day, all indicators of a healthy lifestyle. The primary risk factors of interest in this study were the prevalence of specific genotypes and the adduct ratio, neither of which would be expected to be influenced by differences in educational level of the study participants.

The second outcome of this project was the analysis of SNPs in the genes for four selected estrogen-metabolizing enzymes: cytochrome P450 (CYP)1A1 (I462V), CYP1B1 (V432L),catechol-*O*-methyltransferase (COMT) (V158M) and quinone reductase (NQO1) (P609S). CYP1A1 primarily catalyzes the oxidation of estrone (E_1) and estradiol (E_2) to 2-catechol estrogens [2-$OHE_1(E_2)$], and CYP1B1 predominantly primarily catalyzes the oxidation of E_1 and E_2 to 4-catechol estrogens [4-$OHE_1(E_2)$]. In contrast, COMT and NQO1 are protective enzymes because COMT catalyzes the methylation of catechol estrogens to methoxyestrogens, which cannot be further oxidized, and NQO1 catalyzes the reduction of catechol estrogen quinones to catechol estrogens, thereby preventing the catechol estrogen quinones from reacting with DNA.

Genetic polymorphisms. Genotyping was successful for all but one control who was missing a genotype for both CYP1B1 and NQO1 and one ovarian cancer case missing COMT. No women were homozygous for the CYP1A1 mutant allele and only three were heterozygous for the allele. Therefore, CYP1A1 was not included in the analyses. The three genetic polymorphisms studied here were all in Hardy-Weinberg Equilibrium (HWE) in controls. Because the numbers were small, we also tested for HWE in the combined sample of cases and controls and found that all SNPS were in HWE. Not surprisingly, no differences were seen in allele and genotype frequencies in African American women compared to white women, but the numbers of African American women were small. Table 3 shows the prevalence of alleles and genotypes for each of the three SNPS used in the analyses.

The DNA adduct ratio was increasingly higher in women with one and two high activity CYP1B1 alleles, showing a dose response relationship (Table 3). No such pattern can be seen for COMT or NQO1. To increase the sample size in these categories for subsequent analyses, the alleles were collapsed into two categories such that being either heterozygous or homozygous for the at-risk allele was modeled as being at an increased risk of ovarian cancer. Individually, none of the three SNPs showed significant associations with either the DNA adduct ratio or ovarian cancer. However, in women who were homozygous for the low activity COMT allele, the CYP1B1 high activity allele was associated with a significantly increased DNA adduct ratio (Table 4). The combination of high-risk CYP1B1 and COMT alleles also elevated the odds of having ovarian cancer, although the confidence intervals are wide and the result does not reach statistical significance (Table 4). No significant difference was seen in women who were heterozygous for the COMT low activity allele (Met). Previous studies have also reported negative findings for COMT and risk of ovarian cancer. No studies have assessed NQO1 in relation to ovarian cancer, but larger numbers are needed to assess the NQO1 (Ser) allele in the presence of high CYP1B1 activity and low COMT activity.

Table 3. Frequency of alleles and genotypes and association with DNA adduct ratio.

Allele frequencies	n	DNA adduct ratio (M±SD)	Ovarian cancer Number (%)
CYP1B1			
CC	21	47.5±32.5	9 (27.3)
CG	29	54.7±39.4	14 (42.4)
GG	16	78.1±65.4	10 (30.3)
COMT			
GG	11	61.0±34.2	7 (21.9)
GA	37	47.7±38.4	14 (43.7)
AA	18	75.5±61.7	11 (34.4)
NQO1			
CC	35	62.9±52.8	16 (48.5)
CT	28	55.9±37.6	16 (48.5)
TT	3	22.6±8.00	1 (3.03)
Genotype frequencies	**n**	**DNA adduct ratio (M±SD)**	**Ovarian cancer Number (%)**
CYP1B1			
C	21	47.5±32.5	9 (27.3)
G	45	63.0±50.7	24 (72.7)
COMT			
G	11	61.0±34.2	7 (21.9)
A	55	56.8±48.5	25 (78.1)
NQO1			
C	35	62.9±53.0	16 (48.5)
T	31	52.7±37.1	17 (51.5)

Table 4. Descriptions and t-tests for association between the DNA adduct ratio and having one or two high activity CYP1B1 alleles in the presence of being homozygous for the mutant allele resulting in low COMT activity.

Risk combination	n	Mean adduct ratio (SD)	T-test (p-value)	Ovarian Cancer OR (95% CI)
CYP1B1 GC or GG + COMT AA				
No	52	49.7 (36.8)	-2.43	Reference
Yes	13	91.4 (64.9)	(0.018)	2.84 (0.77-10.4)
CYP1B1 GG + COMT AA				
No	59	51.9 (37.6)	-2.43	Reference
Yes	6	118.5 (79.5)	(0.018)	5.93 (0.65-53.9)

In order to assess the effects of at-risk genotypes, DNA adduct ratio and ovarian cancer in the same model, we modeled successive regressions in a path analysis (Figure 2). The effects were sufficiently strong that a maximum likelihood model converged in only 67 women. The results showed significantly higher DNA adduct ratios in those with one or two deleterious CYP1B1 high activity alleles (Val) and two deleterious COMT low activity alleles (Met/Met). A strong, significant association was seen between the adduct ratio and odds of having ovarian cancer.

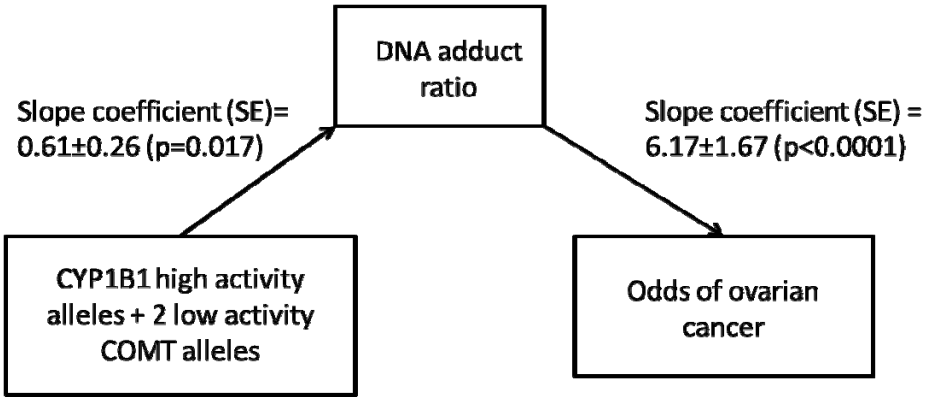

Figure 2. Maximum likelihood path analysis of having polymorphic alleles in CYP1B1 and COMT, the adduct ratio, and having ovarian cancer in 67 women (33 cases and 34 controls).

The DNA adduct ratio may act as an intermediate on the causal pathway between genetic polymorphisms and risk of hormonal cancers. The estrogen quinone resulting from CYP1B1 activity may proceed to adduct formation in the presence of low methylation activity by COMT. A number of studies have examined the effects of combinations of mutant alleles, but have not linked these mutant alleles to the ratio of adduct level to metabolites and conjugates. Having a CYP1B1 Val allele and a COMT Met allele increased the risk of ovarian cancer in a study of 73 cases and 76 controls (OR 2.6, 95% CI 1.2-5.5) [6]. An increased risk of prostate cancer was observed in a study of 1,983 men (1,101 cancer cases and 882 controls) with CYP1B1 Val and COMT Met, when combined, showed a significantly elevated risk of prostate cancer (OR= 1.38, 95% CI 1.11-1.72) [7]. The DNA adduct ratio is a more proximal outcome than ovarian cancer and represents the initiation step in the cancer pathway, making it an early marker of risk for ovarian cancer, as well as other hormonal cancers.

Conclusion

This study continues to be important because of the potential to establish a tool to diagnose ovarian cancer at an early stage. We have completed this seminal study as planned. The number of subjects recruited fell short of our goal of n=50 cases and n=50 controls, but the 34 cases and 36 controls who provided urine samples were sufficient to achieve the primary goal of the study, demonstrating that women diagnosed with ovarian cancer form significantly higher levels of estrogen-DNA adducts than control women not diagnosed with cancer ($p<0.0001$). Analysis of genetic polymorphisms in selected estrogen-metabolizing enzymes was also fruitful. In women who were homozygous for the low activity COMT allele, the CYP1B1 high activity allele was associated with a significantly increased DNA ratio and the odds of having ovarian cancer.

A publication is being prepared to report these findings in the scientific literature. When it has been accepted by a peer-reviewed journal, we will send a copy to you. The results of this study are expected to lead to studies of ovarian cancer prevention by use of specific antioxidants, already being studied for breast cancer prevention.

References

1. Greenlee RT, Murray T. Bolden S, Wingo PA. Cancer statistics, 2000. CA Cancer J Clin 2000;50:7-33.

2. Horner MJ, Ries LAG, Krapcho M, Neyman N, Aminou R, Howlader N, Altekruse SF, Feuer EJ, Huang L, Mariotto A, Miller BA, Lewis DR, Eisner MP, Stinchcomb DG, Edwards BK (eds). SEER Cancer Statistics Review, 1975-2006, National Cancer

Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2006/, based on November 2008 SEER data submission, posted to the SEER web site, 2009.

3. Cavalieri E and Rogan E. Unbalanced metabolism of endogenous estrogens in the etiology and prevention of human cancer. J Steroid Biochem Mol Biol 2011;125:169-80.

4. Gaikwad NW, Muti P, Meza J, Yang L, Pruthi S, Ingle J, Rogan EG, Cavalieri EL. The molecular etiology of breast cancer: evidence from biomarkers of risk. Int J Cancer 2008;122:1949-57.

5. Moorman PG, Calingaert B, Palmieri RT, Iversen ES, Bentley RC, Halabi S, Berchuck A, Schildkraut JM. 2008. Hormonal risk factors for ovarian cancer in premenopausal and postmenopausal women. Am J Epidemiol 2008;167:1059-69.

6. Goodman MT, McDuffie K, Kolonel LN, Terada K, Donlon TA, Wilkens LR, Guo C, Marchand LL. Case-control study of ovarian cancer and polymorphisms in genes involved in catecholestrogen formation and metabolism. Cancer Epidemiol Biomarkers Prev 2001;10:209-16.

7. Cussenot O, Azzouzi AR, Nicolaiew N, Fromont G, Mangin P, Cormier L, Fournier G, Valeri A, Larre S, Thibault F, Giordanella JP, Pouchard M, Zheng Y, Hamdy FC, Cox A, Cancel-Tassin G. Combination of polymorphisms from genes related to estrogen metabolism and risk of prostate cancers: The hidden face of estrogens. J Clin Oncol 2007;25:3596-602.

Appendices

None